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Basic nutritional investigation

Subsarcolemmal and intermyofibrillar mitochondrial responses to short-term high-fat feeding in rat skeletal muscle

Raffaella Crescenzo Ph.D., Francesca Bianco Ph.D., Paola Coppola Ph.D., Arianna Mazzoli Ph.D., Giovanna Liverini Prof., Susanna Iossa Prof. *

Department of Biology, University of Naples- "Federico II", Naples, Italy

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ABSTRACT

Objectives: We assessed the alterations in mitochondrial function in skeletal muscle that were elicited by short-term high-fat feeding in sedentary rats.
Methods: Two groups of rats were pair-fed for 1 wk and received a low-fat or high-fat diet. Body composition, energy balance, and glucose homeostasis were measured. Mitochondrial mass, oxidative capacity, and energetic efficiency as well as parameters of oxidative stress and antioxidant defense were evaluated in subsarcolemmal and intermyofibrillar mitochondria from the skeletal muscle.
Results: Body energy, lipid content, and metabolic efficiency were significantly higher and energy expenditure was significantly decreased among rats that were fed a high-fat diet, as compared with controls. Skeletal muscle mitochondrial energetic efficiency, oxidative capacity for lipid substrates, and antioxidant defense were significantly increased in rats that were fed a high-fat diet as compared with controls.
Conclusions: Acute isocaloric high-fat feeding is able to induce increased phosphorylation efficiency in skeletal muscle subsarcolemmal and intermyofibrillar mitochondria. This modification implies a reduced oxidation of energy substrates that may contribute to the early onset of obesity.

Introduction

It is well known that long-term high-fat (HF) feeding is characterized by enhanced metabolic efficiency that induces obesity and insulin resistance. Changes in whole-body metabolic efficiency reflect changes in single organs and tissues, most likely involving those that are major contributors to the daily metabolic rate, such as the liver and the skeletal muscle [1]. We have previously demonstrated that the administration of an HF diet for 7 wk in sedentary rats elicited not only an increase in whole-body metabolic efficiency (thereby leading to the development of obesity and insulin resistance) but also an impairment of skeletal muscle mitochondria [2]. These results support the postulated link between mitochondrial impairment and insulin resistance in skeletal muscle [3,4]. However, these results were obtained at a point in time when insulin resistance had already been developed, so it was not possible to determine whether mitochondrial impairment was a cause or consequence of impaired insulin signaling.

Because the length of the HF diet treatment is a significant factor in the development of skeletal muscle mitochondrial impairment and insulin resistance in rats, we considered it to be of interest for evaluating alterations in mitochondrial function in skeletal muscle elicited by short-term HF feeding in sedentary rats so that the early effects of high levels of dietary fat on skeletal muscle mitochondria can be detected. To this end, we measured skeletal muscle mitochondrial oxidative capacity and the efficiency of oxidative phosphorylation. Experiments were performed on mitochondria located beneath the sarcolemmal membrane (subsarcolemmal [SS]) or between the myofibrils (intermyofibrillar [IMF]). It is well known that these two mitochondrial populations exhibit different energetic characteristics

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* Corresponding author. Tel.: +39-081-253-8111; fax: +39-081-679-233.
E-mail address: susiossa@unina.it (S. Iossa).

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and therefore can be differently affected by physiological stimuli [5,6]. Because the formation of reactive oxygen species (ROS) via the incomplete reduction of oxygen and the activity of the antioxidant system is an important component of mitochondrial functionality, oxidative damage and antioxidant defense in SS and IMF mitochondria were also determined.

Materials and methods

Animals

The animals that were used in the present study were maintained in accordance with Italian Health Ministry regulations and guidelines for the care and use of laboratory animals. All experimental procedures that involved the use of animals were approved by "Comitato Etico per la Sperimentazione Animale" of the University "Federico II" of Naples, Italy.

Two groups of weight-matched male Sprague Dawley rats were caged singly in a temperature-controlled room (23°C ± 1°C) with a 12-h light/dark cycle and pair-fed 480 kJ of metabolizable energy per d (corresponding to the spontaneous energy intake of rats of similar body weight and age) for a period of 1 wk. One group received a low-fat (LF) diet, and the other group received an HF diet rich in lard. The composition of the diets is reported in Tables 1 and 2.

After 1 wk of isocaloric feeding with LF and HF diets, the two groups of animals were euthanized by decapitation for measurements of body composition and energy balance or for blood collection and skeletal muscle harvesting. At the start of the experimental period, some rats were euthanized for measurements of initial body energy and lipid content.

Body composition and energy balance

The rat carcasses were homogenized, and samples were analyzed for energy content by bomb calorimeter. Total body fat content was measured via the Folch extraction method [7]. Total body water content was determined by drying carcass samples in an oven at 70°C for 48 h. Total body protein content was determined with the use of a formula that related the total energy value of the carcass, the energy derived from fat, and the energy derived from protein [8]; the caloric values for body fat and protein were taken as 39.2 and 23.5 kJ/g, respectively [9]. Energy balance measurements were conducted by the comparative carcass technique, which has been detailed previously [10].

Isolation of skeletal muscle mitochondria and assay of uncoupling protein 3

The hind-leg muscles of the rats were rapidly removed and used for the preparation of homogenates and isolated SS and IMF mitochondria, as previously described [10]. Mitochondrial preparations were obtained by pooling the skeletal muscle taken from four rats. Preliminary experiments have shown that the contamination of SS and IMF mitochondria by other ATPase-containing membranes was lower than 10% and that the protease treatment of SS mitochondria had no effect on state 3 and 4 respiratory activities. The addition of cytochrome c (3 nmol/mg protein) only enhanced state 3 respiration by approximately 25% and 10% in SS and IMF mitochondria, respectively. The determination of uncoupling protein 3 (UCP3) was carried out via Western blot with the use of UCP3 antibody (Millipore Corporation, Billerica, MA, USA), as described previously [11].

Table 1
Composition and energy content of experimental diets

Component (g)	Low-fat diet	High-fat diet
Commercial non-purified diet*	20.7	20.7
Casein	3.0	3.0
Methionine	0.10	0.10
Choline	0.05	0.05
AIN vitamin mix†	0.13	0.13
AIN mineral mix‡	0.5	0.5
Sunflower oil	0.7	0.7
Glucose	10.4	0
Lard	0	4.6
Total (g)	35.6	29.9
Metabolizable energy§ (kJ)	480	480
Metabolizable energy§ (kJ/g)	13.49	16.05
Protein (%)§	23.9	23.9
Lipid (%)§	10.2	46.5
Carbohydrate (%)§	65.9	29.6

AIN, American Institute of Nutrition

* Commercial non-purified diet (4 RF21, Mucedola, Italy) that contains the following components (g/kg): protein, 185; lipid, 30; fiber, 60; and metabolizable energy, 11.20 kJ/g.

† American Institute of Nutrition, 1977.

‡ American Institute of Nutrition, 1980.

§ Estimated by computation with the use of the following values (kJ/g) for energy content: protein, 16.736; lipid, 37.656; and carbohydrate, 16.736.

Table 2
Fatty acid composition (g/100 g fatty acid) of experimental diets*

Fatty acid	Low-fat diet	High-fat diet
4:0-10:0		0.19
12:00		0.19
14:00		1.08
16:00	11.89	22.20
18:00	3.90	14.44
20:00	0.29	0.06
14:01		0.41
16:01	0.55	2.20
18:01	27.65	37.99
20:01	1.17	1.08
22:01	0.83	0.19
18:02	51.22	18.68
18:03	2.50	1.30
SFA (%)	16.08	38.16
UFA (%)	83.92	61.84
MUFA (%)	30.20	41.86
PUFA (%)	53.72	19.98
UFA/SFA (%)	5.22	1.62

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids

* Analyzed by gas chromatography.

Measurements of mitochondrial respiration and parameters of mitochondrial energetic efficiency: degree of coupling, uncoupling effect of fatty acids, and proton leak kinetics

The degree of coupling of oxidative phosphorylation q in the presence of palmitate at a concentration of 45 μM or 65 μM for SS and IMF mitochondria, respectively, as well as state 3 and 4 oxygen consumption, were determined as previously described [12].

Proton leak kinetics were obtained as previously reported [2]. The uncoupling effect of fatty acids was assessed by measuring the decrease in mitochondrial membrane potential as has been described previously [2], after the addition of 45 μM or 65 μM palmitate for SS and IMF mitochondria, respectively. These concentrations were selected to obtain a decrease in membrane potential, which is lower than that obtained during the transition from a state 4 condition to a state 3 condition (about 15–20 mV) in both SS and IMF mitochondria.

Determination of mitochondrial mass and of aconitase and superoxide dismutase–specific activity

Mitochondrial SS and IMF mass was assessed indirectly by two different approaches: (1) by measuring the activity of the mitochondrial marker enzyme citrate synthase (CS) in skeletal muscle homogenates and in isolated mitochondria, in the manner described by Srere [13]; and (2) by evaluating the mitochondrial yield. Mitochondria were solubilized in 1% Triton X-100, and active and total aconitase activities were measured with the methods described by Gardner [14] and Hausladen and Fridovich [15], respectively. Superoxide dismutase (SOD)–specific activity was measured as described by Flohè and Otting [16].

Blood parameters

The blood samples were centrifuged at 1400 × g_{av} for 8 min at 4°C. Plasma was removed and stored at −20°C. Plasma insulin concentrations were measured with the use of enzyme-linked immunosorbent assay kits in a single assay to remove interassay variations (Mercodia AB, Uppsala, Sweden). Plasma glucose and non-esterified fatty acids were measured by colorimetric enzymatic method with the use of commercial kits (Pokler Italia, Genova, Italy, for glucose and Randox Laboratories Ltd, Crumlin, UK, for non-esterified fatty acids). Glucose homeostasis was assessed with the homeostasis model assessment index: (Glucose [mg/dL] × Insulin [mU/L])/405 [17].

Chemicals

All of the chemicals that were used were of analytical grade and were purchased from Sigma (St. Louis, MO, USA).

Statistical analysis

Data are provided as means ± standard errors of the means. Statistical analyses were performed with the use of a two-tailed, unpaired, Student's *t*-test or via non-linear regression curve fitting. Probability values of less than 0.05 were considered to indicate a significant difference. All analyses were performed with the use of GraphPad Prism 4 software (GraphPad Software, La Jolla, CA, USA).

Results

The data regarding body composition at the end of the LF and HF diet treatments are given in Table 3. The two groups of rats had similar body weights, but rats that were fed an HF diet displayed significantly higher body energy and lipid content together with lower protein content as compared with controls. In addition, no differences were found in plasma glucose, insulin, non-esterified fatty acid, and homeostasis model assessment index levels.

At the end of 1 wk of diet treatment, rats that were fed the HF diet exhibited a significantly greater gain in body lipids and energy as compared with controls, whereas the gain in body protein was significantly lower (Table 4). Energy balance data during diet treatment (see Table 4) indicated that, notwithstanding similar metabolizable energy intake between the two experimental groups, the greater gain in body lipids and energy found in rats that were fed the HF diet was the result of lower values of energy expenditure and net energy expenditure (NEE), which can represent the cost of body energy maintenance. Table 4 also shows a significantly higher gross energetic efficiency among rats that were fed the HF diet as compared with controls. It can thus be argued that the spared energy will contribute to enlarged lipid stores in the rats fed the HF diet.

Mitochondrial SS and IMF mass from skeletal muscle at the end of LF and HF diet treatment was indirectly determined by using the mitochondrial marker enzyme CS. At the end of the diet treatment, CS activity per gram of tissue was unchanged in the two experimental groups, both in muscle homogenate and in isolated SS and IMF mitochondria, and no difference was found in CS-specific activity per milligram of protein in SS and IMF mitochondria (Table 5). The yield of SS and IMF mitochondria from the two experimental groups was the same (see Table 5). These results indicate that mitochondrial SS and IMF masses were unchanged in both groups of rats.

Oxidative capacities of SS and IMF skeletal muscle mitochondria were evaluated with the use of NAD, FAD, and lipid substrate (Fig. 1) as follows: 1) glutamate: an NADH-linked substrate that enters through complex I of the respiratory chain; 2) succinate: an FAD-linked substrate that is used to specifically test the tricarboxylic acid cycle and mitochondrial complexes II, III, and IV of the electron transport chain while complex I is inhibited by rotenone; and 3) palmitoyl coenzyme A: a lipid substrate with oxidation that reflects the activity of carnitine palmitoyltransferase I and the intramitochondrial beta-oxidation pathway. At the end of the diet treatment, state 3 respiratory activities with palmitoyl coenzyme A as substrate of IMF (Fig. 1A) and SS (Fig. 1D) mitochondria were significantly higher in rats that were fed the HF diet than in controls. No variations in state 4 respiratory activity (Fig. 1B, E) and respiratory control ratio (RCR) values (Fig. 1C, F) were found in IMF and SS mitochondria at the end of the diet treatment.

We determined the degree of coupling of oxidative phosphorylation, proton leak, and the uncoupling effect of fatty acid palmitate to assess mitochondrial energetic efficiency, which was found to be increased in SS and IMF mitochondria from rats that were fed the HF diet. In IMF and SS skeletal muscle mitochondria, significantly lower values of respiration in the presence of oligomycin were found in the rats fed the HF diet (Fig. 2A), whereas respiration uncoupled by Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) was not affected (Fig. 2B); the values of the degree of coupling were thus significantly higher among rats fed the HF diet as compared with controls (Fig. 2C). The results on mitochondrial proton leak (as assessed by the titration of the steady state respiration rate as a function of mitochondrial membrane potential in SS and IMF skeletal muscle mitochondria) are presented in Figure 3. These

Table 3
Body composition, plasma glucose, insulin, and nonesterified fatty acid levels after 1 wk of feeding with a low-fat or high-fat diet

	Low-fat diet	High-fat diet
Body weight (g)	270 ± 2	274 ± 3
Body energy (kJ)	2214 ± 87	2504 ± 68*
Body lipids (g)	29.7 ± 1.2	40.3 ± 1.1*
Body proteins (g)	41.6 ± 1.2	37.0 ± 1.1*
Body water (g)	180 ± 9	169 ± 10
Insulin (ng/mL)	1.25 ± 0.15	1.30 ± 0.21
Glucose (mg/mL)	214 ± 10	215 ± 8
HOMA index	30 ± 1	31 ± 2
NEFA (mM)	0.10 ± 0.01	0.11 ± 0.01

HOMA, homeostasis model assessment; NEFA, non-esterified fatty acids
Results are given as means ± standard errors of the means of six different experiments
Homeostasis model assessment index was calculated as (Glucose [mg/dL] × Insulin [mU/L])/405
* $P < 0.05$ as compared with the low-fat diet according to a two-tailed, unpaired Student's *t*-test.

Table 4
Body energy balance after 1 wk of feeding with a low-fat or high-fat diet

	Low-fat diet	High-fat diet
Weight gain (g)	50 ± 5	54 ± 2
Metabolizable energy intake (kJ)	3092 ± 98	3050 ± 89
Energy gain (kJ)	700 ± 59	990 ± 48*
Lipid gain (kJ)	490 ± 32	847 ± 48*
Protein gain (kJ)	200 ± 11	143 ± 9*
Energy expenditure (kJ)	2392 ± 51	2060 ± 32*
Gross energetic efficiency (%)	23 ± 2	33 ± 2*
Net energy expenditure (kJ)	1966 ± 49	1745 ± 53*
Net energy expenditure/ME intake (%)	63.6 ± 2.2	60.9 ± 1.5

Results are given as means ± standard errors of the means of six different experiments and refer to the whole period of diet treatment (1 wk)
* $P < 0.05$ as compared with the low-fat diet according to a two-tailed, unpaired Student's *t*-test.

Table 5
Citrate synthase activity and protein yield in intermyofibrillar and subsarcolemmal skeletal muscle mitochondria after 1 wk of feeding with a low-fat or high-fat diet

	Low-fat diet	High-fat diet
Citrate synthase activity μmol/(min × g tissue)		
Homogenate	18.0 ± 0.68	18.6 ± 0.59
Intermyofibrillar	4.93 ± 0.51	4.91 ± 0.33
Subsarcolemmal	1.63 ± 0.11	1.58 ± 0.12
Citrate synthase specific activity μmol/(min × mg protein)		
Intermyofibrillar	2.9 ± 0.13	3.0 ± 0.08
Subsarcolemmal	3.0 ± 0.15	2.8 ± 0.06
Protein yield mg/g tissue		
Intermyofibrillar	1.50 ± 0.13	1.40 ± 0.07
Subsarcolemmal	0.50 ± 0.03	0.51 ± 0.03

Results are given as means ± standard errors of the means of six different experiments

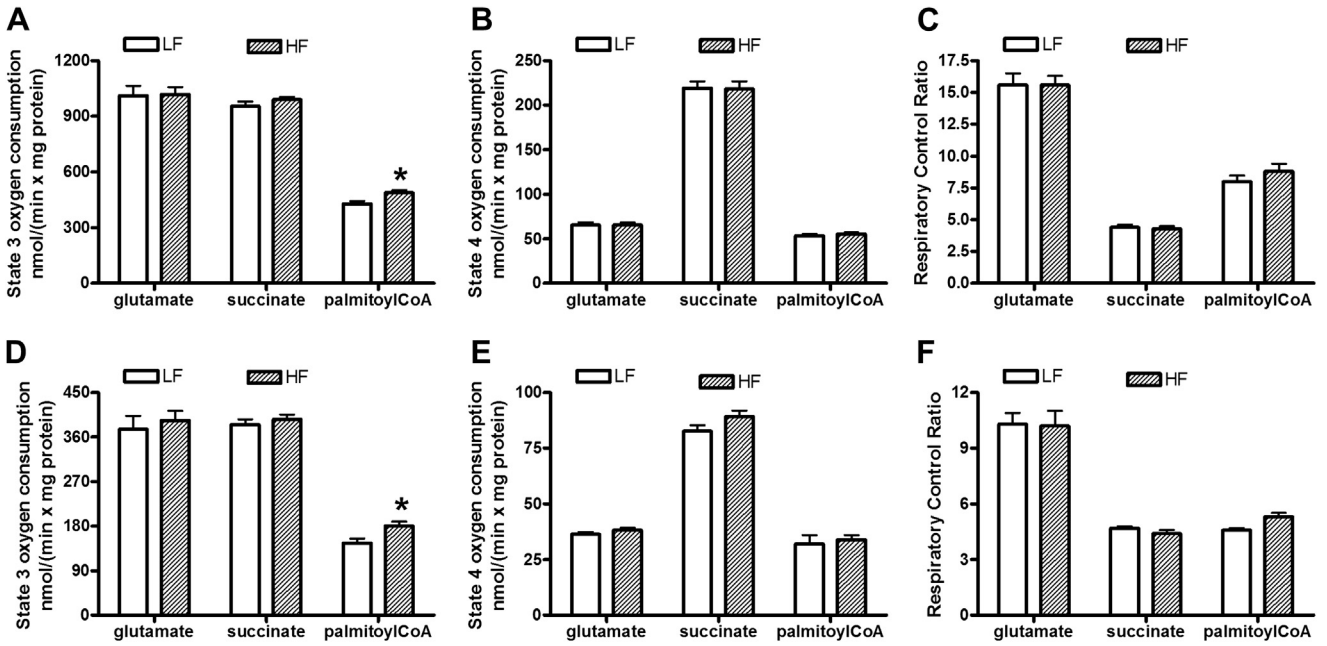

Fig. 1. State 3 respiration, state 4 respiration, and respiratory control ratio in intermyofibrillar mitochondria (A, B, C) and subsarcolemmal mitochondria (D, E, F) after 1 wk of feeding with a low-fat (LF) or high-fat (HF) diet. Results are given as means ± standard errors of the means of six different experiments. $^{*}P < 0.05$ as compared with LF according to a two-tailed, unpaired Student's *t*-test.

titration curves are an indirect measurement of proton leak, because the steady state oxygen consumption rate (i.e., the proton efflux rate) in non-phosphorylating mitochondria is equivalent to the proton influx rate caused by proton leak. The comparisons of these curves (for SS and IMF mitochondria isolated from the two experimental groups at the end of the diet treatment) show that the proton leak significantly decreased in SS (Fig. 3A) and IMF (Fig. 3B) mitochondria from rats fed an HF diet as compared with controls. With regard to the uncoupling effect of palmitate, the decrease in mitochondrial state 4 membrane potential induced by palmitate was found to be significantly lower in IMF and SS mitochondria from rats fed the HF diet as compared with controls (Fig. 3C).

The ratios between active and total aconitase activity and SOD-specific activity were measured and taken as an index of cellular oxidative damage and antioxidant defenses, respectively (Table 6). At the end of 1 wk of diet treatment, as was seen in the IMF mitochondria, rats that were fed the HF diet exhibited a significant increase in the active and total aconitase activity ratio as compared with controls, but there was no change in SOD-specific activity. With regard to SS mitochondria, rats that were fed the HF diet exhibited no change in the active and total aconitase activity ratio, but they did have significantly increased SOD-specific activity. UCP3 protein content as measured by Western blot analysis was found to be significantly higher only in IMF mitochondria from rats fed the HF diet as compared with controls (see Table 6).

Discussion

Evidence is presented here that short-term HF feeding is associated with obesity development without any impairment in glucose homeostasis. In addition, in skeletal muscle, the IMF and SS mitochondrial compartments are characterized by increased oxidative capacity for fatty acids, higher antioxidant defense, and elevated energetic efficiency.

The present results associated with energy balance and body composition indicate that acute exposure (1 wk) to an HF diet—despite similar metabolizable energy intake during the experimental period—induces obesity in rats. These rats were characterized by limited physical activity as a result of their standard housing conditions, and it was shown that rats that were fed the HF diet exhibited higher body energy and lipid levels. One of the causes of the increased energy and lipid gain in rats fed an HF diet could be the reduced de novo lipid synthesis pathway that was previously found in rats fed an HF diet [18,19]. In fact, there is no need for the body to generate long-chain fatty acids when the diet already supplies large amounts of this substrate. The suppression of the de novo lipid synthesis pathway increases the efficiency of nutrient storage in adipose tissue, because there is a high energy cost associated with building long-chain fatty acids from glucose or amino acids [20]. The diminished energy partitioning to protein that was found here also contributes to the excess fat deposition with the HF diet. Despite the same protein intake, rats that were fed the HF diet displayed fat-induced "stunted" growth, probably because of an increase in hepatic gluconeogenesis from amino acids as a result of the lower carbohydrate intake [21]. When this differential "obligatory" energy cost of fat and protein deposition was subtracted, the resulting net energy expenditure was still found to be significantly lower among rats fed an HF diet as compared with controls, thereby indicating that a thrifty energy metabolism is induced early with HF feeding.

One of the possible mechanisms of energy sparing that could be responsible for the thrifty energy metabolism may involve changes in the cellular efficiency of energy handling, especially in tissues with high metabolic activity, such as skeletal muscle [1]. Because more than 90% of cellular energy is produced during oxidative phosphorylation in the mitochondria, changes in mitochondrial mass (that reflect number, size, or both) and functionality could exert profound effects on energetic handling. Changes in mitochondrial protein mass were checked in two

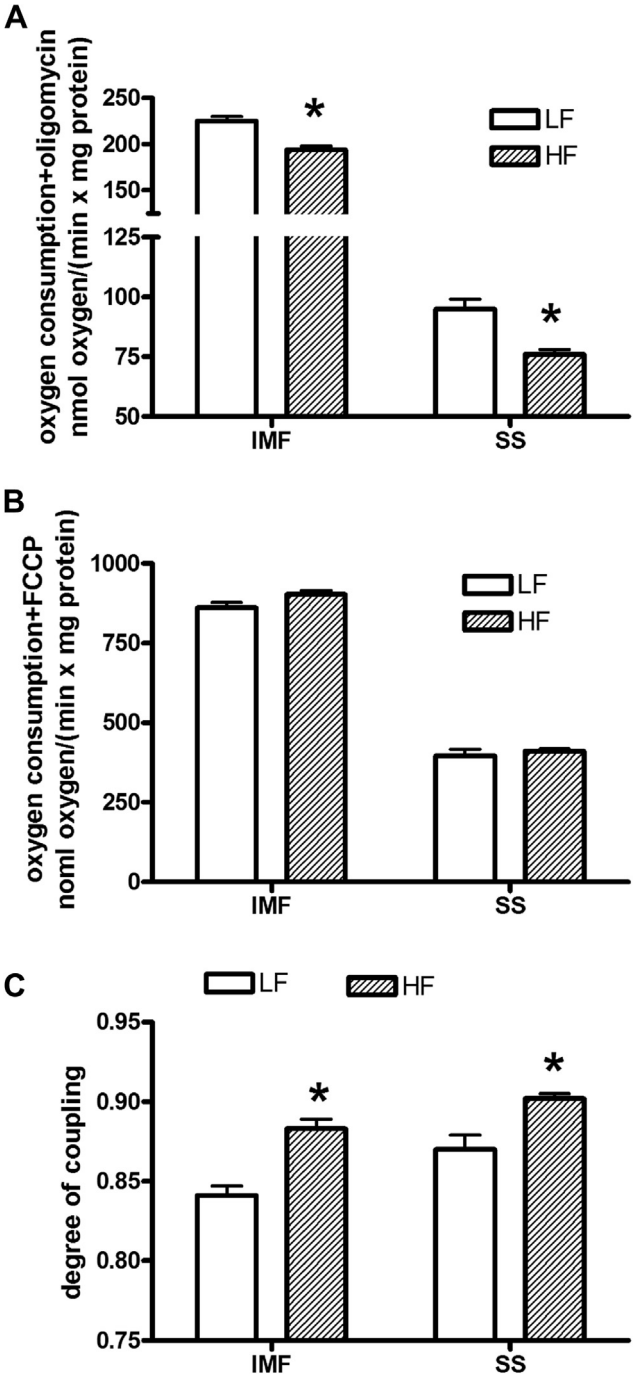

Fig. 2. Oxygen consumption in the presence of oligomycin (A) or uncoupled by FCCP (B) and the degree of coupling values (C) calculated from oxygen consumption in the presence of oligomycin and uncoupled by FCCP in IMF and SS mitochondria after 1 wk of feeding with a low-fat (LF) or high-fat (HF) diet. Results are given as means ± standard errors of the means of six different experiments. $^*P < 0.05$ as compared with LF according to a two-tailed, unpaired Student's *t*-test. *IMF*, intermyofibrillar mitochondria; *SS*, subsarcolemmal mitochondria.

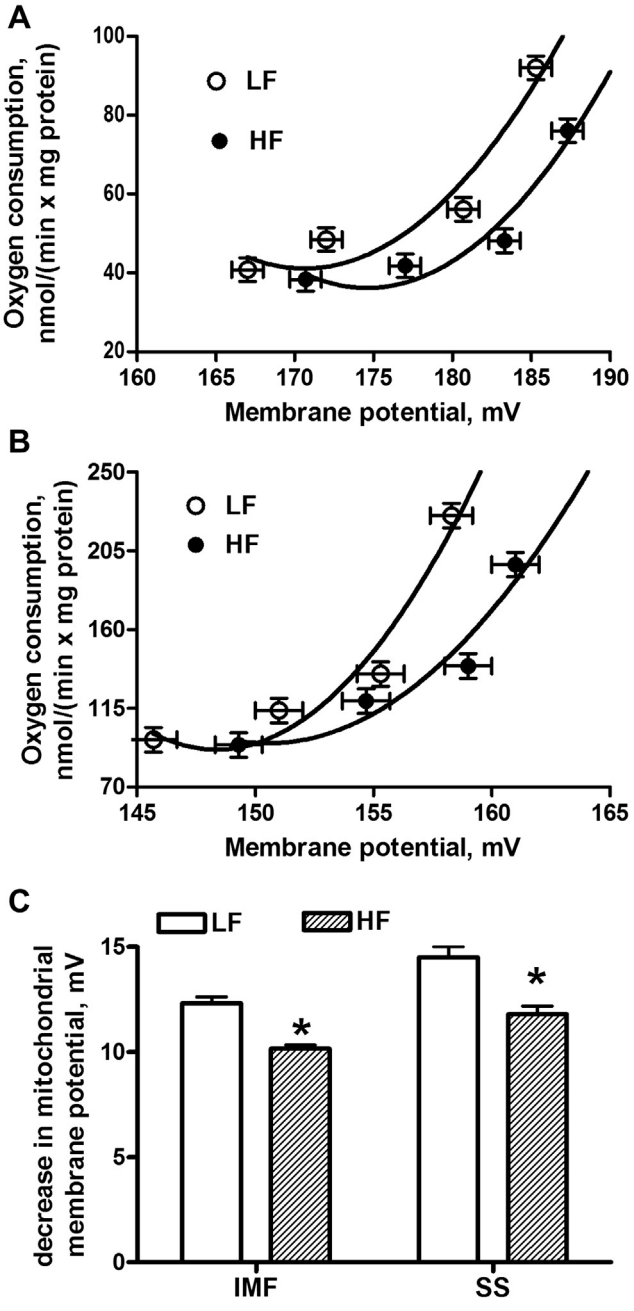

Fig. 3. Kinetics of proton leak in subsarcolemmal mitochondria (A) and intermyofibrillar mitochondria (B) and measurements of the decrease in state 4 membrane potential after the addition of palmitate in subsarcolemmal mitochondria and intermyofibrillar mitochondria (C) after 1 wk of feeding with a low-fat (LF) or high-fat (HF) diet. Results are given as means ± standard errors of the means of six different experiments. In (A) and (B), non-linear regression curve fits demonstrated that the proton leak was significantly lower ($P < 0.05$) in intermyofibrillar and subsarcolemmal mitochondria from rats fed the HF diet as compared with rats fed the LF diet. In (C), $^*P < 0.05$ as compared with LF according to a two-tailed, unpaired Student's *t*-test.

ways: (1) by measuring the activity of mitochondrial marker enzyme CS in skeletal muscle homogenates and in isolated SS and IMF mitochondria; and (2) by evaluating the mitochondrial yield (i.e., mg isolated proteins/g starting wet tissue) in each mitochondrial subpopulation. We found that mitochondrial mass was unchanged in both SS and IMF organelles, thereby indicating that reduced mitochondrial content is not an early effect of HF feeding in rodents; this is in agreement with similar results obtained from humans who were fed a short-term HF diet [22].

Whether defects in mitochondrial fatty acid oxidative capacity are present in the skeletal muscle of rats fed an HF diet is not clear

Table 6
Aconitase, superoxide dismutase–specific activity, and UCP3 protein content in intermyofibrillar and subsarcolemmal skeletal muscle mitochondria after 1 wk of feeding with a low-fat or high-fat diet

	Intermyofibrillar		Subsarcolemmal	
	Low-fat diet	High-fat diet	Low-fat diet	High-fat diet
Active aconitase (mU/mg protein)	50.6 ± 2.2	68.6 ± 3.6*	80.2 ± 2.4	83.1 ± 4.3
Total aconitase (mU/mg protein)	101.7 ± 5.8	110.6 ± 3.8	150.9 ± 7.6	160.0 ± 4.7
Active/total aconitase	0.50 ± 0.02	0.62 ± 0.02*	0.53 ± 0.02	0.52 ± 0.02
Superoxide dismutase (U/mg protein)	28.4 ± 1.1	28.7 ± 0.6	28.6 ± 0.9	36.3 ± 1.2*
UCP3 protein content (arbitrary units/mg protein)	18.5 ± 1.7	28.2 ± 1.6*	17.7 ± 1.7	16.4 ± 1.6

Results are given as means ± standard errors of the means of six different experiments For UCP3 protein content, values are the means ± standard errors of the means of four different experiments

* $P < 0.05$ as compared with the low-fat diet according to a two-tailed, unpaired Student's *t*-test.

because of conflicting results [3,10,23–25]. The present findings indicate that mitochondrial fatty acid oxidative capacity is increased in both SS and IMF mitochondria. The increased capacity for fatty acid oxidation could be an early compensatory response to elevated fatty acid substrate availability. We also detected parameters of oxidative phosphorylation efficiency, such as mitochondrial proton leak, palmitate uncoupling effect, and the degree of coupling in isolated SS and IMF mitochondria. The results clearly indicate that the acute administration of an HF diet increases oxidative phosphorylation efficiency in both mitochondrial populations so that fewer fuels are oxidized to obtain the same amount of ATP. A decreased substrate burning by skeletal muscle is in agreement with the decrease in energy expenditure found here among rats fed an HF diet, given that skeletal muscle accounts for about 30% of whole-body energy expenditure [1]. Our data thus suggest that variations in skeletal muscle mitochondrial energetic efficiency contribute to thrifty energy metabolism elicited by HF feeding. It could be hypothesized that altered mitochondrial energetic efficiency, being present at a time point when insulin sensitivity is normal, precedes and could contribute to the blunted insulin response that is typical of longer-term HF feeding [2,19]. The present finding of an unaltered homeostasis model assessment index after 7 d of HF feeding is in agreement with a recent study [26] that demonstrated that, under conditions of hyperinsulinemic-euglycemic clamps, both whole-body and skeletal-muscle insulin-stimulated glucose use were not altered by isocaloric HF feeding for 11 d.

The present data regarding a lower proton leak and the palmitate uncoupling effect are suggestive of increased mitochondrial ROS production. In fact, ROS production by the mitochondrial respiratory chain is higher when membrane potential increases [27,28], thereby leading to an increased probability for electrons to react directly with dioxygen and to form superoxide and related ROS [29]. For these reasons, we also assessed the oxidative status of SS and IMF skeletal muscle mitochondria, and we did not find signs of oxidative damage. The absence of oxidative damage among rats that were fed the HF diet can be explained by considering that in these rats we found increased UCP3 protein content in IMF mitochondria and increased SOD activity in SS mitochondria. UCP3 provides a first line of defense against ROS, which are linked to insulin resistance [30]. UCP3 has also been proposed as a translocator of fatty acid peroxides from the inner to the outer membrane leaflet, thus preserving macromolecules from being oxidized by very aggressive fatty acid peroxides [31]. The previously mentioned finding of increased SOD activity in SS mitochondria is in agreement with the normal insulin sensitivity that was also found. In fact, SS mitochondria provide energy for membrane-related processes, including signal transduction, ion exchange, substrate transport, and substrate activation [32]; these are steps that are clearly relevant to insulin action. It has recently been shown that the overexpression of mitochondrial SOD in skeletal muscle from rats fed an HF diet improved muscle glucose uptake through an altered redox state [33].

Conclusions

Acute HF feeding is able to increase oxidative phosphorylation efficiency in skeletal muscle SS and IMF mitochondria. This modification could have detrimental metabolic effects by causing energy sparing that contributes to the early onset of obesity. A deleterious consequence of the increased mitochondrial energetic efficiency in skeletal muscle IMF and SS mitochondria could be the reduced oxidation of fatty acids and consequent triglyceride accumulation in the skeletal muscle, which may later generate muscle insulin resistance.

Acknowledgments

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